EXPERIMENTS WITH HISTAMINASE

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By mechanical stimulation of the normal human skin a substance is released which has a physiological effect on the small blood vessels very similar to, or identical with, that of histamine. Thomas Lewis has called this the "H-substance" (1).

The urticarial wheal and flare resulting from the intradermal injection of histamine appears to be identical with the lesions of spontaneously occurring urticaria and with the positive skin reactions produced by intradermal injection of the specific allergen in patients with hay fever, asthma, atopic eczema and certain other clinical manifestations of allergy.

The pathological physiology of these diseases may be partially explained by assuming that, as a result of the reaction between the specific allergen and the antibodies of the sensitized tissues, one or more "H-substances" are liberated which produce vascular dilatation, transudation of plasma and cells, possibly smooth muscle contraction and other effects.

There are several reasons, however, for questioning the proposition that the hypothetical "H-substance" of these conditions is identical with histamine. Among these may be mentioned:

1. The failure of Abramson to obtain whealing substance from allergic wheals by iontophoresis although he was able, by the same procedure, to recover whealing substance (histamine) from histamine wheals (2).

2. The experiments of Bowman and Walzer which indicate that tissue refractoriness resulting from intradermal injection of histamine is not identical with the refractoriness resulting from intradermal injection of specific allergen (3).

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3. The histologic differences (slight) between allergic wheals and histamine wheals as reported by Kline, Cohen and Rudolph (4).

The enzyme, histaminase, is present in many tissues of the body and is obtained commercially especially from intestinal mucous membrane. Experiments have demonstrated that it is capable of destroying histamine *in vitro*. The treatment of allergic and other diseases with histaminase is based upon the theory that the hypothetical "H-substance" of these conditions is chiefly or entirely histamine and that, by administration of histaminase the concentration of this enzyme in the tissues may be increased to such an extent that the histamine liberated or produced in the tissues will be destroyed before it reaches an effective concentration.

In order to investigate certain aspects of the action of histaminase in allergic conditions the following experiments were performed with a preparation of histaminase now available commercially.

1. INCUBATION OF HISTAMINE AND HISTAMINASE IN VITRO

A quantity of histaminase said to be capable of destroying 1.0 mg. of histamine (one unit) was mixed in a test tube with 0.9 cc. physiological solution of sodium chloride and 0.1 cc. of a 1:200 solution of histamine (Ergamine acid phosphate) containing 0.5 mg. histamine was added.

A histamine control was prepared containing 0.9 cc. physiological solution of sodium chloride and 0.1 cc. of 1:200 solution of histamine.

A histaminase control was prepared containing the same quantity of histaminase named above plus 1.0 cc. physiological solution of sodium chloride.

After incubation at 37°C. for 24 hours intradermal tests were made by injecting 0.02 cc. from each of the three tubes into a presumably normal person, with the following results:

Histamine plus histaminase	no wheal
Histaminase control	no wheal
Histamine control	typical histamine wheal.

This experiment demonstrated that the preparation of histaminase being used was potent, i.e., capable of destroying histamine in vitro.

2. THE ACTION OF HISTAMINASE ON HISTAMINE INJECTED INTRADERMALLY

(a) *Injection of histamine into skin sites previously injected with histaminase*

Histaminase, 0.1 unit in 0.1 cc. solution was injected intradermally into each of five separate locations in a presumably normal person.

Histamine, 1:10,000, 0.02 cc. (0.002 mg. histamine) was injected intradermally into the first location immediately after the injection of histaminase; into the second, five minutes later; into the third, one hour later, and into the fourth, 24 hours later. The fifth location remained as a control.

Histamine was also injected intradermally in the same quantities and at the same times into skin sites previously injected with physiological solution of sodium chloride, 0.1 cc., at the same times that the five other skin sites were injected with histaminase.

In each instance the histamine, when injected into a site previously injected with histaminase, produced a wheal and erythema which did not differ appreciably from the corresponding controls. The quantity of histamine injected was only $\frac{1}{25}$ the minimal quantity which the histaminase previously injected was capable of destroying *in vitro,* as demonstrated in experiment # 1.

This experiment demonstrated that histaminase injected into the tissues was not capable of destroying histamine sufficiently rapidly to abolish its action when the latter was injected intradermally. This result might readily have been expected in view of the known slow action of histaminase in vitro—an action analogous to digestion and fermentation.

(b) *Injection of histaminase into skin site previously injected with histamine*

Histamine, 1:10,000, 0.02 cc. (0.002 mg. histamine) was injected intradermally and immediately thereafter histaminase, 0.10 unit in 0.1 cc. solution was injected into the same location.

The resulting wheal and erythema appeared the same as that produced by the same quantity of histamine followed by physiological solution of sodium chloride, 0.1 cc., intradermally. Histaminase alone, 0.1 unit in 0.1 cc. solution was also injected intradermally for comparison.

This experiment, supplementing 2 (a), demonstrated that histaminase injected into the tissues did not destroy histamine sufficiently rapidly to abolish its action when the latter was injected intradermally.

3. THE ACTION OF HISTAMINASE ON THE HYPOTHETICAL "H-SUBSTANCE" OF ALLERGIC REACTIONS

An experiment analogous to 2 (a) was performed as follows: Five skin sites on a presumably normal, non-ragweed sensitive person were passively sensitized by intradermal injection (0.1 cc. each) of serum from a ragweed sensitive hay fever patient. Two days later histaminase, 0.1 unit in 0.1 cc. solution was injected into each site.

Ragweed pollen extract, 1:10,000, 0.02 cc. (2 Noon pollen units) was injected intradermally into the first site immediately after the injection of histaminase; into the second, 5 minutes later; into the third one hour later, and into the fourth 24 hours later. The fifth site remained as a control.

Ragweed pollen extract was also injected intradermally in the same quantities and at the same times into skin sites previously sensitized and subsequently injected with physiological solution of sodium chloride, 0.10 cc., at the same times that the five other skin sites were injected with histaminase.

In each instance the ragweed pollen extract, when injected into a site previously injected with histaminase, produced a wheal and erythema which did not differ appreciably from the corresponding controls.

This experiment demonstrated that histaminase injected into the tissues was not capable of destroying the hypothetical "H-substance" of hay fever (and presumably of related forms of allergy), sufficiently rapidly to abolish its action when it was produced or liberated rapidly in the tissues.

4. THE EFFECT OF HISTAMINASE ON THE SYMPTOMS OF HAY FEVER

A ragweed hay fever patient was allowed to inhale (in January when no pollen was in the air) a small quantity of Dwarf Ragweed pollen from the end of a tooth pick. An attack of sneezing, nasal congestion and discharge followed within a few minutes. He was then given histaminase, 15 units three times per day for seven days. On the seventh day he was again allowed to inhale the pollen in approximately the same amount. *The nasal symptoms which followed within a few minutes were no different than those previously produced.*

5. THE EFFECT OF HISTAMINASE ON FACTITIOUS URTICARIA (DERMOGRAPHISM)

A patient with definite dermographism, in whom stroking the skin with a blunt instrument (tooth pick) resulted in a definitely elevated, linear wheal, 5 to 6 mm. in diameter, was given histaminase by mouth, 15 units three times per day. The skin was tested by stroking before administration and again on the fifth day of treatment.

The wheals produced after administration of histaminase appeared just the same as the controls previously produced.

COMMENT

In experiments 2 and 3, involving observations concerning the combined effects of two dissimilar substances injected intradermally, consideration must be given to the possibility of differences in the rate of diffusion of these substances through the tissues. If, for example, histaminase and histamine were injected at approximately the same time, and if histamine had a more rapid diffusion rate than histaminase, it would be possible for histamine to come in contact with tissues containing little or none of the injected histaminase. And conversely, if histaminase diffused much more rapidly than histamine, the concentration of histaminase at the injection site, at the time of a later injection of histamine, might be much less than expected. An attempt to overcome these possible difficulties was made by injecting histamine at several time intervals after the injection

of histaminase and by injecting histaminase (shortly) after the injection of histamine.

The injection of histamine (or allergen) into a wheal "mechanically" produced by the previous injection of as much as 0.1 cc. of fluid, gives rise to some difficulty in interpretation of results. By careful comparisons with the controls, including measurements of the wheals and flares, these difficulties may be overcome.

SUMMARY AND CONCLUSIONS

1. The enzyme histaminase, as previously demonstrated, destroys histamine when incubated with the latter *in vitro*.

2. Histaminase injected into the tissues does not destroy histamine sufficiently rapidly to abolish its action when the latter is injected intradermally.

3. Histaminase injected into the tissues does not destroy the hypothetical "H-substance" of hay fever (and presumably of related forms of allergy) sufficiently rapidly to abolish its action when the latter is produced as the result of an intradermal injection of specific allergen in a passively sensitized skin site.

4. The administration of histaminase by mouth to a hay fever patient did not prevent the development of typical hay fever symptoms following inhalation of the specific pollen.

5. The administration of histaminase by mouth to a patient with factitious urticaria (dermographism) did not prevent the development of wheals following stroking of the skin.

REFERENCES

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